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THE OPSONIC INDEX AND VACCINE THERAPY OF PSEUDODIPHTHERIC OTITIS.*

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ALMOST a year and a half ago, in the course of experiments made by Dr. Horton and myself to determine the nature of a certain immune serum, it was found that opsonins for pseudodiphtheria bacilli are present in human blood and in the blood of some of the lower animals, and that these bodies can be increased by repeated injections of cultures of the bacilli.[1] The pseudodiphtheria strains used in these experiments belong to the variety usually known as the "Ruediger bacillus," and are distinguished from other varieties by the fact that they are virulent for guinea-pigs and that the serum of rabbits immunized against them is bacteriolytic for this variety but not for other pseudodiphtheria bacilli. The serum of human beings was found to contain opsonin for these bacilli and also the serum of the dog, goat, rabbit, and guinea-pig. Rabbits and goats were immunized by injections of broth cultures of one of the strains, and the resulting serum, which was strongly bacteriolytic and agglutinative, had also increased greatly in opsonic content. "Nine experiments were made with leucocytes of lower animals and 40 with human leucocytes. Without exception, in all of these experiments the presence of our immune serum favored phagocytosis of this group of bacilli, but had no effect on phagocytosis of *B. diphtheriae*, or of six strains of ordinary pseudodiphtheria bacilli. There was practically very little phagocytosis in the tubes containing no serum, there was a varying amount in the tubes with homologous serum, but in the experiments with the virulent pseudodiphtheria bacilli there was always a marked increase in the amount of phagocytosis in the tubes with immune serum as compared with normal rabbit serum." In these experiments the serum, washed corpuscles, and bacterial suspension were used in the

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[1] Hamilton and Horton, *Jour. Infect. Dis.*, 1906, 3, p. 128.

proportion of one part each of the first two to two parts of the last. As a thick bacterial supsension was used and the tubes incubated for 45 minutes, the number of bacteria taken up was large. The results were stated in numbers representing the average number of bacilli ingested by 40 leucocytes. Normal rabbit serum being taken as the control, we should have an index of 4.9 for the immune rabbit serum when used with human leucocytes. The opsonin in the immune serum was specific for these bacilli, *B. diphtheriae* and the ordinary varieties of pseudodiphtheria bacilli were not affected by it, the index for the former being 0.85 and for the latter (an average of eight counts) 0.8. Immune goat serum also contained increased opsonin for the Ruediger bacillus but not for other varieties of pseudodiphtheria bacilli nor for *B. diphtheriae.*

To carry on similar experiments with the serum of human beings instead of serum of the lower animals it would be necessary to have under observation cases in which the Ruediger bacillus had been found, but none was at hand. Instead was studied a class of patients which were easily available, namely patients suffering from a form of otitis media (see p. 326 of this number) in which pseudodiphtheria bacilli are found in large quantities. As pointed out in the following article, these bacilli resemble the Ruediger bacillus in many ways, but differ from it in that, while most strains of the latter are non-fermenters, the former ferment dextrose, some of them ferment saccharose (Group 1), and those which do not ferment saccharose ferment maltose (Group 2). The chief distinction, however, lies in the fact that the serum of rabbits immunized against the Ruediger bacillus contains bacteriolysin, agglutinin, and opsonin for the Ruediger bacillus, but acts simply like normal rabbit serum toward both types of the pseudodiphtheria bacilli of otitis media, while conversely the serum of a rabbit immunized against Group 1 of the latter contains bacteriolysin, agglutinin, and opsonin for the active strains of this group, but not for the Ruediger bacillus.

The results of the opsonin tests made with the immune Ruediger serum have been given. The following are the indices obtained with the serum of a rabbit immunized against a strain of Group 1 (fermenting saccharose). The serum of normal rabbits was used as control.

Group 1	4.0
Group 1	2.2
Group 1	2.1
Group 2 (fermenting maltose)	0.8
Group 2	1.1
Ruediger Bacilllus	0.9

The Ruediger bacillus was found only three times in an examination of 142 cases of otitis media, but the two other varieties were found 51 times. Seventy-two per cent of the cases of scarlatinal otitis media contained them. The arguments as to the etiological significance of this organism are given elsewhere, and it is only necessary to state here that the results of the opsonic tests made with serum from these patients go to strengthen the belief that the bacilli in question are the causative agents in certain cases of otitis media.

Twenty-two cases of otitis media from which the pseudodiphtheria bacillus had been isolated were selected and their opsonic index to their own strain was determined. Seventeen were scarlet-fever patients, two measles, and three were cases of chronic recurrent otitis, who were at the time suffering from an attack of the disease. As control, seven cases of scarlet fever and measles were taken, two of which had otitis media, but the pus in these cases yielded streptococci only. Finally 16 examinations of serum from normal persons were made. For these control tests, five of the strains of pseudodiphtheria bacilli isolated from otitis media cases were used, three belonging to Group 1, two to Group 2.

Table 1 gives the indices obtained from the normal cases, from the measles and scarlet-fever cases without pseudodiphtheria infection, and from the cases with pseudodiphtheria bacilli in the pus from the ear. Only two of the pseudodiphtheria cases had indices within the normal limits, the others were all very much below. In two of the cases in this table it was possible to make but one test, in eight more, from two to five, could be made, and in the remaining 12 the examination could be carried on for a period covering several weeks. The index of these patients was found to have quite a wide range and a certain correspondence between the clinical symptoms and the index could be noted. Thus one case, a chronic recurrent purulent otitis, had an index of 0.3 during an attack and of 1.2 at the time of discharge. Another chronic case had an index of 0.4 at the beginning and of 1.3 at the end of the attack. A case of acute scarlatinal otitis, in which the index was taken three times, began with 0.8, fell to 0.2, and was up to 1.0 at the end. As would be expected the indices of the patients showed a much wider range than those of normal individuals. The following are the lowest and highest indices in four cases:

TABLE 1.

THE OPSONIC INDEX FOR PSEUDODIPHTHERIA BACILLI IN PSEUDODIPHTHERIC OTITIS, MEASLES, AND SCARLET FEVER, AND IN HEALTH.

Otitis Media	Measles and Scarlet Fever	Normal Persons
0.11 (scarlatinal)	0.7	0.6
0.11 "	0.9	0.7
0.17 "	0.9 streptococcal ear	0.8
0.19 "	0.9 " "	0.81
0.19 "	1.0	0.9
0.2 "	1.0	0.9
0.2 "	1.16	1.0
0.2 "		1.0
0.26 (measles)	0.95 Average	1.0
0.3 (scarlatinal)		1.09
0.3 "		1.1
0.3 "		1.1
0.3 (chronic recurrent)		1.1
0.38 (measles)		1.25
0.38 (scarlatinal)		1.3
0.38 "		1.3
0.4 (acute non-scarlatinal)		
0.4 (scarlatinal)		0.99 Average
0.43 "		
0.46 "		
0.7 "		
1.3 (acute non-scarlatinal)		
0.35 Average		

Lowest	Highest
0.2	2.1
0.11	1.6
0.19	2.4
0.39	2.0

Those cases, which were followed for several weeks, showed sometimes a persistently low index and sometimes one with variations corresponding to the subsidence or exacerbation of the symptoms. Two instances of low index are given in Charts 1 and 2. The case illustrated in Chart 1 had a profuse suppuration which persisted up to the time of dismissal from the hospital. The case illustrated in Chart 2 was not nearly so severe a case and there was a decided improvement in the condition of the ear before she left the hospital. In Charts 3 and 4 there is more variation in the indices and the clinical notes show that the index rose or fell before the change in the condition which the rise or fall foreshadowed. Thus in the case illustrated in Chart 3 there was a marked improvement in the condition coincident with the

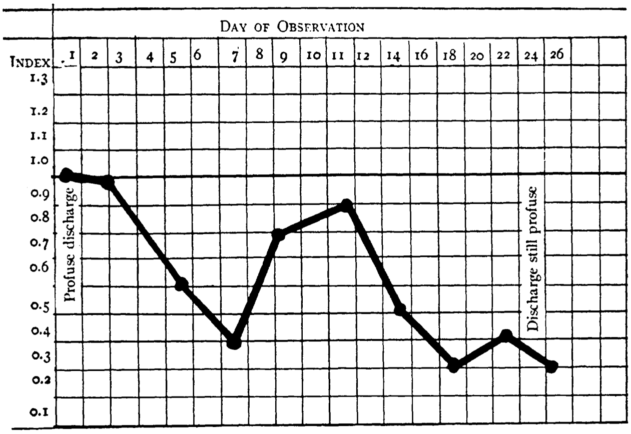

CHART 1.—The opsonic index for pseudodiphtheria bacillus (Group 1) in acute otitis.

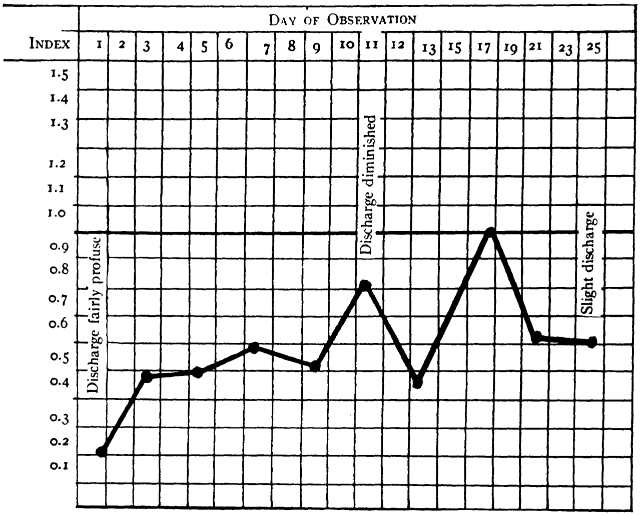

CHART 2.—The opsonic index for pseudodiphtheria bacillus (Group 1) in acute otitis.

higher index. When the blood was taken on the 24th day it was supposed that the index would prove normal, and the drop here was not explained until, on the 27th, the ear was found to be discharging profusely again. Chart 4 is that of a child who began with a serious suppuration, improved, and then relapsed and was dismissed with a low index, the ear still suppurating. On the other hand, Chart 5

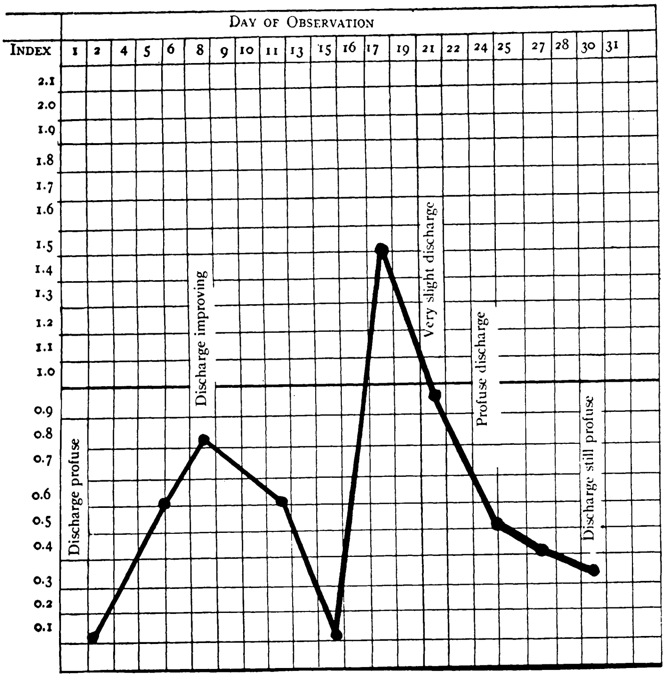

CHART 3.—The opsonic index for pseudodiphtheria bacillus (Group 2) in acute otitis.

shows a curve with great variations and yet the course of the disease was fairly constant, the suppuration was always quite profuse. When the child left the hospital the index was very high, but the ear was not entirely well.

The study of these indices affords a strong argument in favor of the pyogenic character of the pseudodiphtheria bacillus. Those cases of otitis media which yielded cultures of pseudodiphtheria bacilli had—with the exception of two cases in each of which but one examination was made—very low indices to these bacilli. The cases of measles and scarlet fever which had no suppuration in the ear or in which there was a streptococcal infection of the ear, had indices to the pseudodiphtheria bacilli which averaged those of the normal persons examined—0.95 and 0.99. The fact that the variations in the index of these cases corresponded often to the variations in the clinical symptoms also points to the fact that the pseudodiphtheria bacilli was the cause of the suppuration.

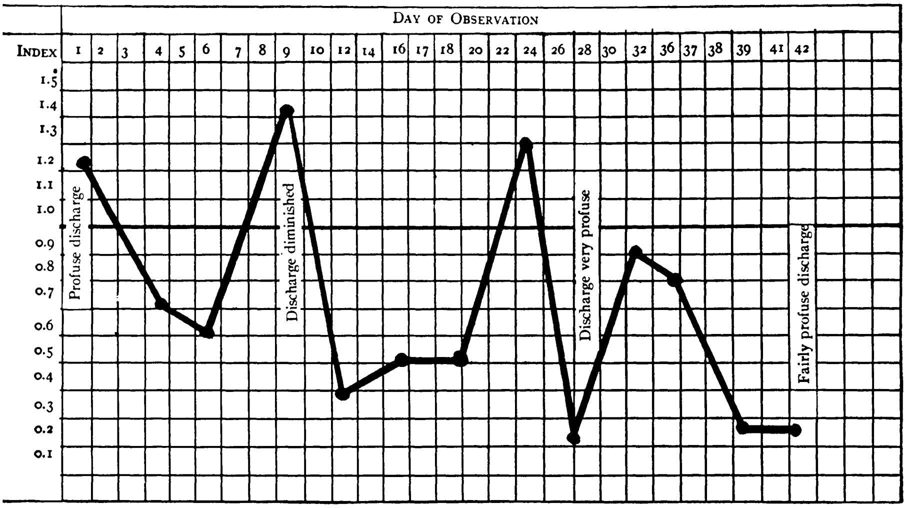

CHART 4.—The opsonic index for pseudodiphtheria bacillus (Group 1) in acute otitis.

The opsonin for these bacilli appears to be specific. Patients suffering with streptococcal complications were selected and their index to the streptococcus and to the pseudodiphtheria bacillus taken with the following result:

	Streptococcus	Pseudodiphtheria bacilli
J. P.	1.4	0.98
M. C.	0.5	0.9
K. G.	1.3	0.29 (Pseudodiphtheric otitis)
A. M.	0.9	0.11 (Pseudodiphtheric otitis)

In one instance the streptococcus index was followed for a long period by Dr. Tunnicliff and the pseudodiphtheria index by myself, the child having a streptococcal suppuration in the cervical glands and pseudodiphtheria bacilli in the pus from the ear. The two curves when superimposed (Chart 6) show that a high index for one organism did not mean a high one for the other, a circumstance that indicates two kinds of opsonin.

As it had been shown that injections of the Ruediger bacillus increases the opsonin in the serum of rabbits and goats, it seemed probable that an analogous phenomenon could be produced in human beings by injections of the bacillus isolated from the suppurating ears, and that the condition might possibly be improved by this increase of the specific opsonin for these bacilli. Seven cases of profuse suppurative scarlatinal otitis media were selected for the study of this point. One child had had discharge for nine weeks, the other 6 for periods varying from 3 to 10 days. It would have been very much more satisfactory to have carried out these experiments with old, chronic, and obstinate cases which had not yielded to ordinary methods of treatment, for in such cases it would be comparatively easy to show whether or not vaccine therapy had any value. In acute scarlatinal otitis media we are dealing with a process which is subject to sudden, spontaneous cure, so that it is practically impossible to demonstrate that any remedial agent influences its course. But the difficulty of controlling dispensary patients, the impossibility of making as many and as frequent tests as would be necessary, forced me to turn to the material in the hospital and to take acute instead of chronic cases.

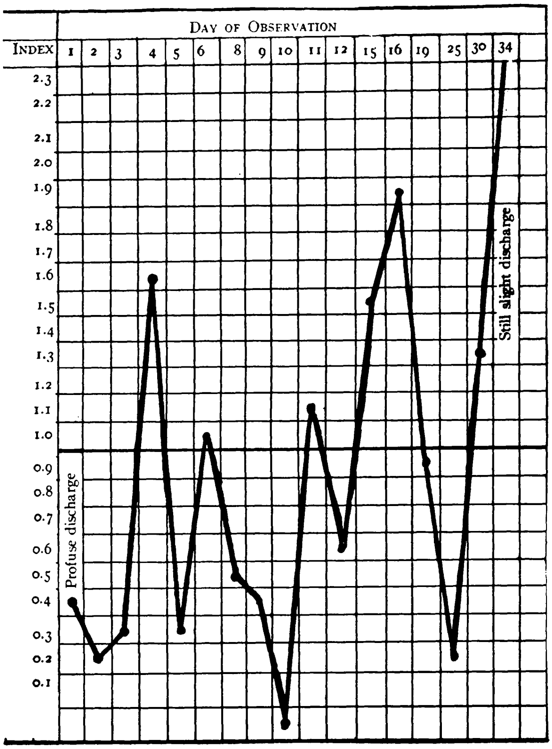

Chart 5.—The opsonic index for pseudodiphtheria bacillus (Group 2) in acute otitis.

The vaccine for these injections was prepared by suspending in normal salt solution a twenty-four-hour old agar-slant culture and heating it to 65° for forty minutes. In all cases the absolute sterility of the vaccine was demonstrated by cultural methods. The dose given at first was one-half of this amount, then it was increased to three-quarters and then to a whole culture, but as the effect of the larger doses was no greater than that of the small it seemed best to return to the original dose and one-half a slant culture was given regularly. One case had a bacillus of Type 2, growing rather scantily on agar and the dose in this instance was a full slant culture.

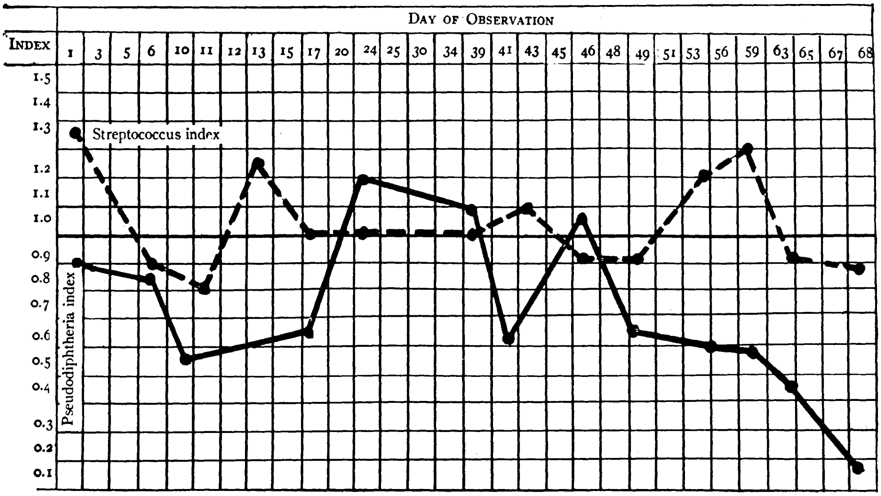

CHART 6.—The opsonic indices for pseudodiphtheria bacillus (Group 1) and staphylococcus in pseudodiphtheric otitis and cervical streptococcal infection.

No local reaction followed the injection of these vaccines and no constitutional reaction, so far as could be discovered. The first case had to be abandoned after two injections because the child developed laryngeal diphtheria and a very weak heart which made it inadvisable to persist in an experimental treatment. The first injection had raised the index from 0.25 to 1.6, the second from 0.5 to 1.15. No change was noticed in the condition of the ear.

The second case was given two injections and then was dismissed from the hospital. The suppuration was still fairly profuse, the index had risen only to 1.15 and at dismissal was 0.7. The third case was more satisfactory. This child had had a profuse suppuration of the middle ear for nine weeks. She was given two injections, the treatment extending over 13 days. At the end of that time she was

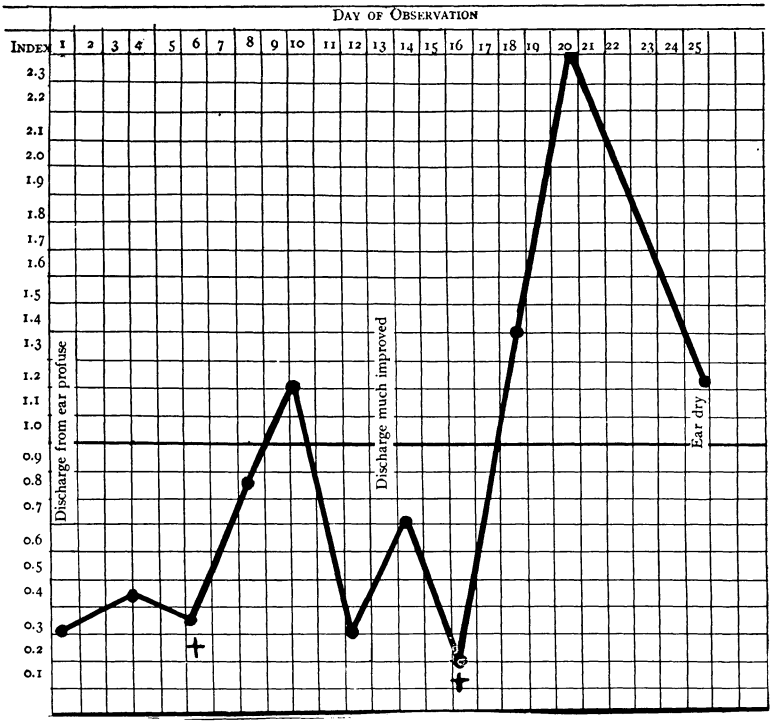

CHART 7.—The opsonic index in pseudodiphtheric (Group 1) otitis treated with autogenous vaccine. + = injection of vaccine.

dismissed with only a slight, thin discharge from the ear. Her index rose from 0.3 to 1.3 after the first injection. The charts of the other four are given (Charts 7, 8, 9, and 10). It will be seen that in all, the injections were followed by a more or less pronounced rise of the patient's index, often without any preliminary negative phase. In all four cases the clinical symptoms also improved and two of the patients (Charts 7 and 10) had no discharge when they left the hospital, the other two (Charts 8 and 9) had a slight discharge. However, at the same time and in the same two wards four other children, all with the same form of otitis media, made as rapid and as good recovery as these four, without any injections of vaccine.

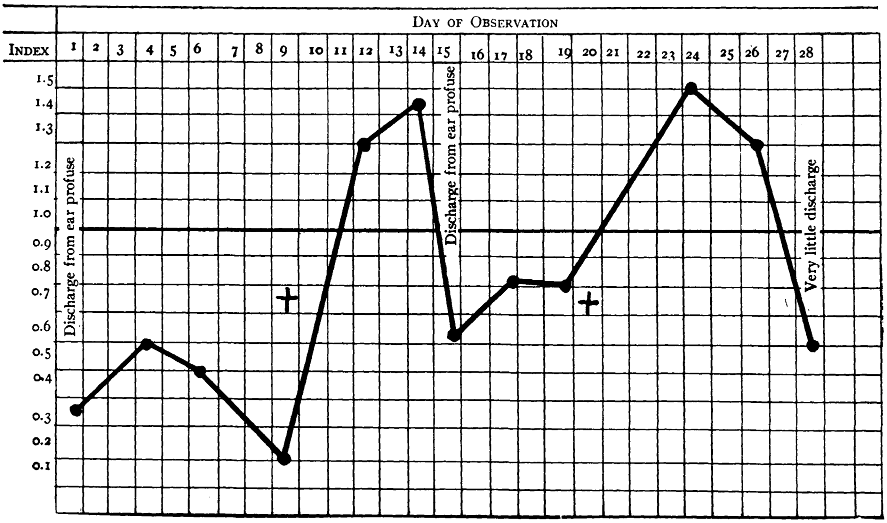

CHART 8.—The opsonic index in pseudodiphtheric (Group 1) otitis injected with autogenous vaccine. + = injection of vaccine.

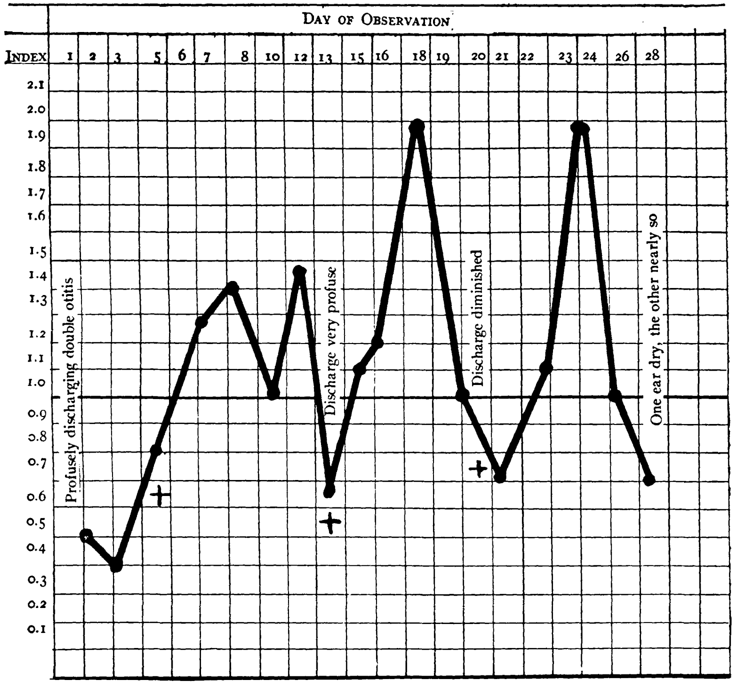

CHART 9.—The opsonic index in pseudodiphtheric (Group 2) otitis injected with autogenous vaccine. + = injection of vaccine.

It would, of course, be unwarranted to claim that the value of vaccine treatment is proved by events in these cases because, as I have said, acute suppurative otitis media so often heals rapidly under no particular treatment. But it seems reasonable to believe that patients who have a low opsonic index to the organism found in the pus from the ear will be benefited by a treatment which results in the raising of their index. The impression obtained so generally of the effects of vaccine therapy of staphylococcal infections warrants us in supposing

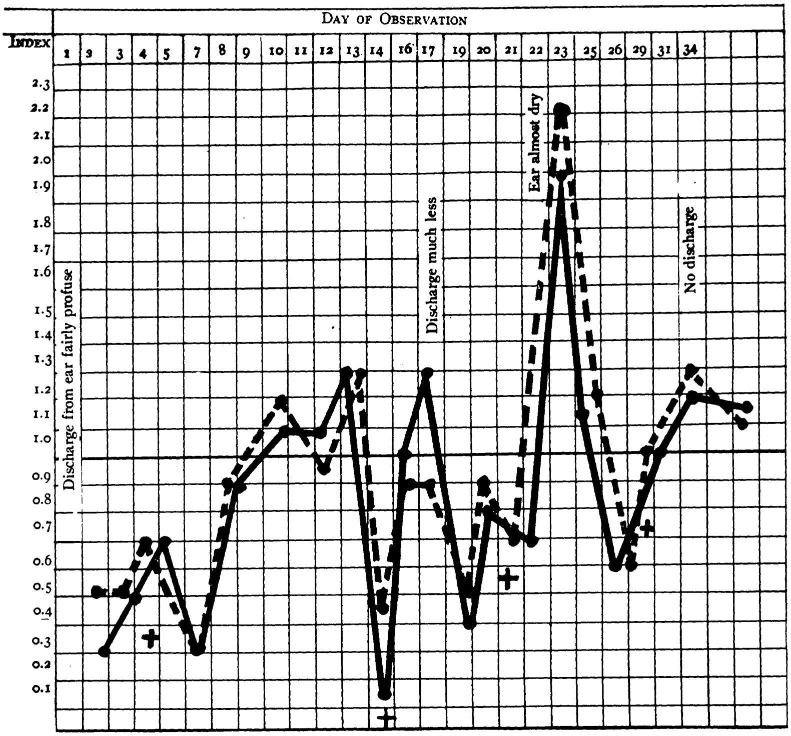

CHART 10.—The opsonic index in pseudodiphtheric (Group 1) otitis injected with autogenous vaccine.

NOTE.—The solid line represents the opsonic index as usually estimated—relation of the average number of bacteria taken up under the influence of the patient's serum per leucocyte to the average number of bacteria taken up per leucocytes in the pooled, normal serum—and the dotted line the proportion of phagocyting leucocytes in the patient's serum as compared with those in the pool. These two indices were estimated in the other cases also, the second serving as a control for the first. Wide variations seem to depend largely on an irregular distribution of the bacilli and with care the two indices usually come out very much alike. In all these experiments equal parts were used of washed blood, bacterial suspension, and serum.

this to be true. It seems probable, then, that chronic suppurative otitis media may prove a fruitful field for this form of treatment.

SUMMARY.

Cases of otitis media which have pseudodiphtheria bacilli as the predominating organism in the pus from the ear have usually a low opsonic index for this bacillus.

In certain cases in which repeated examinations are made the index is found to cover a wide range, and the changes in the index often correspond to changes in the clinical symptoms.

By injections of dead cultures of the homologous strain the patient's opsonin to that strain can be increased. No ill effect follows such injections and an apparent improvement has resulted in several cases. The opsonin in the blood of these patients is specific for the variety of pseudodiphtheria bacilli to which the patient's strain belongs. This constitutes a strong proof that the pseudodiphtheria bacillus plays an etiologic rôle in such cases.